Endophytes: a rich source of functional metabolites

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1 Introduction

An endophyte is a bacterial (including actinomycete) or fungal microorganism, which spends the whole or part of its life cycle colonizing inter- and/or intra-cellularly inside the healthy tissues of the host plant (Fig. 1), typically causing no apparent symptoms of disease. The endophytic population of a given species varies from several to a few hundreds of bacterial and fungal strains. Endophytes can be isolated from mildly surface-sterilized plant tissues and cultivated on nutrient agar (Fig. 2). The relationship between the endophyte and its host plant may range from latent phytopathogenesis to mutualistic symbiosis. Presumably owing to their specialised niches, no substantial body of work has accumulated since the first discovery of endophytic fungus in darnel in 1904. However, much renewed

13 Chlorinated metabolites
14 Others
15 Comment
16 References

1 Introduction

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attention is now being paid to the chemistry and bioactivity of endophyte metabolites, and to endophytic biodiversity and related ecological functions. This review, beginning with a brief survey of the biological aspects of endophytes, describes endophyte metabolites characterized before 2000.

2 Biological survey of endophytes

2.1 Distribution and biodiversity

Almost all vascular plant species examined to date were found to harbor endophytic bacteria and/or fungi. Moreover, the colonization of endophytes in marine algae, mosses and ferns has also been recorded. As a matter of fact, endophytes are important components of microbial biodiversity. Commonly, several to hundreds of endophyte species can be isolated from a single plant, among them, at least one species showing host specificity. The environmental conditions under which the host is growing also affect the endophyte population, and the endophyte profile may be more diversified in tropical areas.

2.2 Origin and evolution

Some phytopathogens in the environment are of endophyte origin. Many innocuous fungal endophytes are causative phytopathogens which may cause infectious symptoms when the host plant is aged and/or stressed. On the other hand, during the long co-evolution of the phytopathogen and its host plant, an endomorphic mutant may result from balanced antagonism and/or gene mutation. Dual cultures of the host calli and endophytes demonstrated that both the endophytes and the host calli excrete metabolites toxic to each other. Further investigation led to the development of a hypothesis that the endophyte–host interaction could be a balanced pathogen–host antagonism. Freeman and Rodriguez found that a naturally occurring nonpathogenic endophytic mutant developed from the mutation of a single locus in the genome of the wild-type Colletotrichum magnus, a pathogen causing anthracnose in cucurbit plants. This mutant is able to grow systemically inside the host plant without pathogenic symptoms, but retaining wild-type levels of in vitro sporulation, spore adhesion, appressoria formation, infection and host specificity.

The Acremonium (asexual fungi now reclassified in the genus Neotyphodium Glenn, Bacon and Hanlin) endophytes, which usually inhabit tall fescue, perennial ryegrass (Lolium perenne L.), and many cool-season grasses, are considered mutualistic symbionts of the host grasses. The grass and the endophytic fungus are so intimately associated that they act ‘as a single organism’. And, indeed, some of these endophytic Neotyphodium species can only spread by infecting seeds from the mother plants (Fig. 3).

2.3 Physiological and ecological roles

Endophytes colonizing inside plant tissues usually get nutrition and protection from the host plant. In return, they confer profoundly enhanced fitness to the host plants by producing certain functional metabolites.

2.3.1 Growth promotion of the host plant

Endophyte-infected plants often grow faster than non-infected ones. This effect is at least in part due to the endophytes’ production of phytohormones such as indole-3-acetic acid (IAA), cytokines, and other plant growth-promoting substances, and/or partly owing to the fact that endophytes could have enhanced the hosts’ uptake of nutritional elements such as nitrogen and phosphorus. A culture broth of Colletotrichum gloeosporioides, an endophyte fungus of Artemisia annua L., has also been found in our laboratory to be able to promote the growth of the host callus (Fig. 4, unpublished work).

2.3.2 Improvement of the hosts’ ecological adaptability

Certain endophytes improve the ecological adaptability of hosts by enhancing their tolerance to environmental stresses.
and resistance to phytopathogens and/or herbivores including some insects feeding on the host plant. Endophyte-infected grasses usually possess an increased tolerance to drought and aluminium toxicity. Furthermore, some endophytes are able to provide the host plant with protection against some nematodes, mammal and insect herbivores as well as bacterial and fungal pathogens. Some endophytes are capable of enhancing the hosts’ allelopathic effects on other species co-growing nearby, usually being competitor(s) for the nutrition and the space. This could be the reason why some plants with special endophytes are usually competitive enough to become dominant species in successional fields.

3 Alkaloids

3.1 Amines and amides

Amines and amides are quite common secondary metabolites of *Acremonium* endophytes, the anamorphic *Epichloë* species reclassified as genus *Neotyphodium* Glenn, Bacon and Hanlin. These endophytic fungi inhabit tall fescue, perennial ryegrass and many cool-season grasses. Peramine, a pyrrolopyrazine alkaloid, was characterized both in culture and in planta from *Neotyphodium coenophialum*, *N. lolii*, *Epichloë festucae* and *E. typhina* present in the stem and leaf of tall fescue, ryegrass and other grasses. This secondary metabolite was shown to be toxic to insects (extremely to Argentine stem weevil) without any harmful impact on mammals. Biosynthetically proline, arginine and a methyl donor have been proposed to be involved in the biosynthesis of the compound. Peramine is currently accepted as a unique chemical marker for the fungi in the *Neotyphodium*/*Epichloë* complex.

The ergot alkaloids were the second group of amine and amide alkaloids discovered in cultures of *Neotyphodium* endophytes, all being also characterized previously from ergot sclerotia. In addition, these metabolites were demonstrated later to be neurotoxic to insects and mammal herbivores. Ergovaline and other structurally related ergopeptines have been assumed to be most likely responsible for the toxicosis of livestock after consuming endophyte-infected tall fescue. Biosynthesis of ergot alkaloids such as ergovaline is better understood with the ergot fungus *Claviceps purpurea*. Tryptophan and a mevalonic acid derivative dimethylallyl diphosphate were demonstrated to be precursors of these metabolites. The novel amide was characterized as a ras-farnesyltransferase inhibitor from the culture broth of an endophytic *Phomopsis* sp. Furthermore, three new cytotoxic cytochalasans, together with the known metabolite cytochalasin E, were purified from the culture broth of *Rhinocladiella* sp., an endophyte present in the perennial twining vine of *Tripterygium wilfordii*.
sp. originating from twigs of *Salix gracilostyla* var. *melano-stachys.* In disk diffusion assays, this metabolite was shown to be antibacterial against *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella gallinarum*, and antifungal against the human pathogenic yeast *Candida albicans*. 1-**N**-Methylalbonoursin, an unusually fluorescent and weakly antibiotic alkaloid of prokaryote origin, was also isolated from the liquid culture of an *Acremonium*-like *Streptomyces* sp., an endophyte living in perennial ryegrass seedling tissues. Biosynthetically, the diketopiperazine skeleton of the compound was demonstrated to originate from leucine and phenylalanine. Most recently, a tetramic acid analog cryptocin, a potent antifungal against *Pyricularia oryzae* and other phytopathogens, was characterized from the culture of an endophytic fungus *Cryptosporiopsis cf. quercina* present in the inner bark of the stems of *Tripterygium wilfordii*.

### 3.2 Indole derivatives

Indole alkaloids such as chanoclavine, agroclavine and elymoclavine, previously characterized from ergot sclerotia, were reisolated from a culture of *Neotyphodium* endophytes. They are toxic to some insects and mammals. A non-tremorgenic lolilline and tremorgenic indoleterpenes paxilline, lolitrem A, B and E, and terpendole C were detected not only in cultures of *N. lolii* and *E. festucae*, but...
also in the endophyte-infected plants *Lolium perenne* and *Festuca* spp. These tremorgenic metabolites were ascertained as the causative agents of ryegrass staggers in livestock. Attention to the biosynthesis of the lolitrems and paxilline demonstrated that geranylgeranyl pyrophosphate and tryptophan were primary precursors while alkaloids were isolated from *L. perenne* infected with *N. lolii* and the finding confirmed the proposal that lolitriol is the biosynthetic precursor of lolitrem A, B and E (Fig. 6) by Munday-Finch. A metabolic grid for the biosynthesis of lolitrems was proposed as in Fig. 6 by Munday-Finch. Besides grass endophytes and *Aspergillus flavus*, tremorgenic paspalitrems A, B and C can also be produced by *Phomopsis* sp., an endophytic fungus isolated from the living bark of *Cavendishia pubescens*. Some endophytes can produce plant hormones with an endoindole framework. The growth-promoting phytohormone indole-3-acetic acid (IAA, 42) was reisolated from cultures of a root-associated endophytic bacterium *Azospirillum brasilense* SP 7, the tall fescue fungal endophyte *Acremonium coenophialum*, and *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. Together with IAA and indole-3-acetonitrile, cytokinins could also be produced by an endophytic strain of *Hypoxylon serpens* isolated from tobacco. The alternative production of endophytes for phytohormones is assumed to be related to the plant growth-promoting effect allowed by the endophyte infection. On the other hand, a new indole derivative 6-isoprenylindole-3-carboxylic acid 44 was characterized recently from the *A. annua* endophyte *Colletotrichum* sp. It shows moderate antibacterial activity against the Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea* and the Gram-negative bacterium *Pseudomonas* sp. Furthermore this new product is also inhibitory to the growth of some crop phytopathogenic fungi *Phytophthora capsici*, *Rhizoctonia cerealis* and *Geosminomyces graminis* var. *triticum*.6

### 3.3 Pyrrolizidines

Lolines 45–51, saturated aminopyrrolizidine alkaloids, were exclusively found in endophyte-infected grasses such as *Festuca arundinacea* (infected with *N. coenophialum*) and *F. pratensis* (with *N. uncinatum*). Surprisingly, these alkaloids could be detected neither in endophyte cultures nor in non-infected grasses. Lollines are potent broad-spectrum insecticides, acting both as metabolic toxins and feeding deterrents depending on the specific insect species. Unlike ergot and indole diterpene alkaloids, these loline derivatives are much less toxic to mammals although some biological activity in small mammals and mammalian tissues has been reported. Ecologically, certain loline analogs have been demonstrated to contribute to the allelopathic properties of host grasses. The biosynthetic pathway of loline alkaloids is not to date very clear. However, the pyrrolizidine ring system of lolines could originate from spermidine.

In addition to loline 45, *N*-methyloline 47, *N*-formyloline 50 and *N*-acetylolline 51, a new 5,6-dehydro-*N*-acetylolline 52 was isolated from endophyte-infected *Festuca argentina*. Injection of these compounds into mice allowed no obvious toxicity confirming that they were independent of the toxicity of the endophyte-infected plant.

### 4 Steroids

Along with ergosterol 53, 3β,5α,6β-trihydroxyergosta-7,22-diene 54, 3β-hydroxyergosta-5-ene 55, 3-oxoergosta-4,6,8(14),22-tetraene 56, 3β-hydroxy-5α,8α-epidioxyergosta-6,22-diene 57, 3β-hydroxy-5α,8α-epidioxyergosta-6,9(11),22-triene 58 and 3-oxoergosta-4-ene 59, two new steroids, 3β,5α-di-hydroxy-6β-acetoxyergosta-7,22-diene 60 and 3β,5α-di-hydroxy-6β-phenyl-
acetoxyergosta-7,22-diene 61 were characterized from the liquid culture of an fungal endophyte Colletotrichum sp. of A. annua. Metabolites 55, 56, 60 and 61 were shown to be antifungal against some crop pathogens Gaeumannomyces graminis var. tritici, Rhizoctonia cerealis, Helminthosporium sativum and Phytophthora capsici.**

5 Terpenoids
Terpenoids have often been isolated from some endophyte cultures originating from a variety of host plants. Those identified so far are mainly sesqui- and diterpenes, some of which are partly degraded.

5.1 Sesquiterpenes
2α-Hydroxydimeninol 62, 63 and pestalotiopsins A–C 64–66 are sesquiterpenes characterized from endophytic Pestalotiopsis spp. associated with T. brevifolia.65-68 In particular, the new sesquiterpene 63 is a highly functionalized humulane derivative, the first of fungal origin. Heptelidic acid 67 and hydroheptelidic acid 68 isolated from Phyllosticta sp., an endophytic fungus of

Abies balsamea, have been shown to be toxic to spruce bud worm (Choristoneura fumiferana) larvae. Two new benzofuran-carrying normonoterpenes derivatives 69 and 70, toxic to spruce bud worm larvae and/or cells, have been characterized from a culture of an unidentified endophytic fungus obtained from wintergreen Gaultheria procumbens. Some of the first reported sesquiterpenes produced by fungal endophytes were chokols A–G 71–77, which were isolated from Epichloë typhina on Phleum pratense and were found to be fungitoxic to the leaf spot disease pathogen Cladosporium phlei. Obviously, chokols A, F and G were partly degraded.

5.2 Diterpenes

Two new insect toxins 78 and 79 of a pimarane diterpene framework were isolated from the broth of an unidentified
endophyte from a needle of the balsam fir *Abies balsamea*.

Subglutinol A 80 and B 81, immunosuppressive but noncytotoxic, were produced by *Fusarium subglutinans*, an endophytic fungus from the perennial twining vine *Tripterygium wilfordii*.

Guanacastepene 82, a novel diterpenoid produced by an unidentified fungus from the branch of *Daphnopsis americana* growing in Guanacaste, Costa Rica, was shown to be antibacterial against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*.

Taxol 83, originally characterized from the inner bark of the Pacific yew, *Taxus brevifolia*, is an efficacious anticancer diterpene found in extremely small quantities in slowly growing *Taxus* species. Its unique mode of action, of preventing the depolymerization of tubulin during the processes of cell division, made it a huge success in both clinic and market. However, the source of Taxol is a frustrating problem all over the world owing to the difficulty and unacceptably low yield in its total synthesis.

Insecticidal rugulosin 94 was characterized from *Hormonema dematioides*, an endophytic fungus of balsam fir. From cultures of an unidentified endophyte obtained from an eastern larch (*Larix laricina*) needle, 8,1',5'-trihydroxy-3',4'-dihydro-1'-(2,4')binaphthalenyl-1,4,2'-trione 95 was characterized as a toxin to spruce budworm larvae. A highly hydroxylated quinone altersolanol A 96, characterized from phytopathogenic *Alternaria* spp., was reisolated from an endophytic *Phoma* sp. with its antibacterial activity disclosed.

Three new phenylpropanoids 104–105 and lignan 106 were characterized from stromata of *Epichloë typhina* on *Phleum*.
Interestingly, coniferin 107 and syringin 108, two monolignol glucosides produced by the host plant, were ascertained to be specifically recognized by the endophytic Xylariaceae species as chemical signals during the establishment of fungus–plant interactions.

Peptides
Leucinostatin A 109, an oligopeptide with phytotoxic, anticancer and antifungal properties characterized originally from *Penicillium lilacinum*, was reisolated from culture of *Acremonium* sp., an endophytic fungus from *Taxus baccata*. This mycotoxin causes necrotic symptoms in many non-host plants presumably because they cannot transform it into the much less toxic leucinostatin A-β-di-O-glucoside as can the host plant *T. baccata*. The cyclopeptides echinocandins A 110, B 111, D 112 and H 113, produced by *Aspergillus rugulosus* and *A. nidulans* var. *echinulatus*, were reisolated from endophytic *Cryptosporiopsis* sp. and *Pezicula* sp. in *Pinus sylvestris* and *Fagus sylvatica* and shown to be antimicrobial. Crypto-candin 114, a cyclopeptide with potent antifungal activities, is a metabolite of endophytic *Cryptosporiopsis* cf. *quercina* of redwood.

Phenol and phenolic acids
Phenol and phenolic acids, frequently detected in cultures of endophytes, often have pronounced biological activities. 2-Hydroxy-6-methylbenzoic acid was isolated from endophytic *Phoma* sp. and shown to be antibacterial. 2-Methoxy-4-hydroxy-6-methoxymethylbenzaldehyde, produced by a tree endophyte *Pezicula* sp. strain 553, was shown to be antifungal by the bioautography assay against phytopathogen *Cladosporium cucumerinum*. Five known antifungal phenolic acids (p-hydroxybenzoic acid, p-hydroxyphenylacetic acid, tyrosol, cis- and trans-p-coumaric acids) were isolated from stromata of *E. typhina* on *P. pratense* together with compounds 104–106. From *Colletotrichum gloeosporioides*, an endophytic fungus of *P. pratense*, 94 Interestingly, coniferin 107 and syringin 108, two monolignol glucosides produced by the host plant, were ascertained to be specifically recognized by the endophytic Xylariaceae species as chemical signals during the establishment of fungus–plant interactions. 95

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Artemisia mongolica, a new antimicrobial tridepside colletotric acid 115 was characterized in our laboratory. Furthermore, two isomeric novel tridepsides cytonic acids A 116 and B 117 were reported as human cytomegalovirus (an ubiquitous opportunistic pathogen) protease inhibitors from the culture of the endophytic fungus Cytonaema sp. isolated from Quercus sp. 118

12 Aliphatic compounds

Four antifungal aliphatic compounds 118–121 were characterized from stromata of E. typhina on P. pratense. From an endophyte of the eastern larch, a novel ester metabolite 122 was isolated as antibacterial agent against Vibrio salmonicida, Pseudomonas aeruginosa and Staphylococcus aureus. Phomodiol 123 and phomopsolide B 124, metabolites of endophytic Phomopsis spp. present in the genus Salix and non-willow plants, may serve as potential markers for taxonomy of these fungi. 119

13 Chlorinated metabolites

Three chlorinated metabolites from endophytic fungi were reported including an insect-toxic heptelidic acid chlorohydrin 125, and the two antimicrobial and algicidal compounds (−)-mycorrhizin A 126 and (+)-cryptosporiopsin 127. They were isolated from cultures of balsam fir needle endophyte Phyllosticta sp. strain 76, tree endophytes Pezicula sp. and P. livida strain 1156, respectively. Compounds 125 and 126 possess sesquiterpene skeletons, the latter being partly degraded.

14 Others

A new antifungal pentaketide 128 was recently characterized from a Fusarium sp., an endophytic fungus living in the interior part of Selaginella pallescens stem. Other macromolecules such as sulfated oligosaccharides were reported to be capable of mediating the interaction between a marine red alga and its endophyte. Two novel antitumor metabolites sequoiatones A 129 and B 130 were isolated from an endophytic fungus Aspergillus parasiticus of redwood. Three new monoterpenes, C-methylated acetogenins 131–133, were produced by Pestalotiopsis spp., endophytic fungi of Taxus brevfolia, which were shown to be of chemotaxonomic significance. Two possible pathways related to the biosynthesis of these metabolites from certain polyketide precursors were also put forward. In addition to gamahorin, three new antifungal metabolites gamahonolide A 134 and B 135 and 5-hydroxy-4-phenylfuran-2(5H)-one 136 were characterized from stromata of E. typhina on P. pratense. Chaetomelic acids A 137 and B 138 originally isolated from the culture of an endophytic Chaetomella acutisea (MF5686) were found to be specific inhibitors of farnesyl-protein transferase (FPTase).

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As a poorly investigated store of microorganisms ‘hidden’ within the host plants, endophytes are obviously a rich and reliable source of bioactive and chemically novel compounds with huge medicinal and agricultural potential. Further research at the molecular level in this field is clearly required for a better understanding of the host–endophyte interaction which may lead to a quicker recognition of genetically particular and/or host gene carrying endophytes.

15 Comment

Like other microorganisms invading plant tissues, endophytes produce extracellular hydrolyases as a resistance mechanism to overcome attack by the host against pathogenic invasion and/or to get nutrition from the host. Such enzymes including pectinases, esterases, cellulases and lipases, proteinase, 1,4-glucan lyase and phosphatases have been documented with different endophytes. Enzymatic activities closely related to the host-specificity of the endophytes were demonstrated.

The action of these enzymes gives rise to the possibility that the ‘genetic recombination’ of the endophyte with the host may occur in evolutionary time. This could be the reason why some endophytes can produce some phytochemicals originally characteristic of the host. The extended significance of the endophyte with the host may of the endophytes were demonstrated.

16 References
